CLAIMS

1. An isolated or purified nucleic acid wherein said nucleic acid is selected from the group consisting of:
   a. SEQ ID N°1;
   b. Nucleic acid having a sequence fully complementary to SEQ ID N°1;
   c. Nucleic acid having at least 90% sequence identity after optimal alignment with a sequence defined in a) or b);
   d. Nucleic acid that hybridizes under stringent conditions with the nucleic acid defined in a) or b).

2. A nucleic acid fragment comprising at least 8 to 2000 consecutive nucleotides comprised in at least one nucleic acid according to claim 1.

3. The nucleic acid fragment according to claim 2, characterized in that it is susceptible to be used as a probe or a primer specific of SEQ ID N°1.

4. The nucleic acid fragment according to claim 2, selected from the group consisting of : SEQ ID N°17, SEQ ID N°18.

5. The nucleic acid fragment according to claim 2, characterized in that it is obtained by specific amplification of SEQ ID N°1 with the pair of primers SEQ ID N°17 and SEQ ID N°18.

6. The nucleic acid fragment according to claim 2 wherein said nucleic acid fragment is:
   - specifically deleted from the genome of *Mycobacterium tuberculosis,* excepted in *Mycobacterium tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome; and,
   - present in the genome of *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG.*

7. The nucleic acid fragment according to claim 2 or 6 selected from the group consisting of :

a) SEQ ID N°4;

b) Nucleic acid having a sequence fully complementary to SEQ ID N°4;

c) Nucleic acid having at least 90% sequence identity after optimal alignment with a sequence defined in a) or b);

d) Nucleic acid that hybridizes under stringent conditions with the nucleic acid defined in a) or b).

8. A nucleic acid fragment comprising at least 8 to 2000 consecutive nucleotides of at least one nucleic acid according to claim 7.

9. The nucleic acid fragment according to claim 2 or 8, characterized in that it is susceptible to be used as a probe or a primer specific of SEQ ID N°1 and SEQ ID N°4.

10. The nucleic acid fragment according to claim 9, selected from the group consisting of : SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16.

11. A nucleic acid fragment according to claim 9, characterized in that is obtained by specific amplification of SEQ ID N°1 or SEQ ID N°4 with one pair of primers choosed in the group consisting of SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16.

12. The nucleic acid fragment according to claim 9, characterized in that it is obtained by specific amplification of SEQ ID N°1 or SEQ ID N°4 with the pair of primers SEQ ID N°13 and SEQ ID N°14.

13. The nucleic acid fragment according to claim 9, characterized in that it is obtained by specific amplification of SEQ ID N°1 or SEQ ID N°4 with the pair of primers SEQ ID N°15 and SEQ ID N°16.

14. The isolated or purified nucleic acid according to claim 1 wherein said nucleic acid comprises at least a deletion of a nucleic acid fragment according to any of claims 6, 7 and 8.

15. An isolated or purified polypeptide encoded by the nucleic acid according to any of claims 1, 2, 6, 7, 8 and 14.

16. The polypeptide according to claim 15 selected among polypeptides with sequence SEQ ID N°6, SEQ ID N°8, SEQ ID N°10, SEQ ID N°12, SEQ ID N°22 and fragments thereof.

17. An isolated or purified nucleic acid encoding a polypeptide according to claim 16.

18. The isolated or purified nucleic acid according to claim 17, wherein said nucleic acid is selected among :

- SEQ ID N°5 encoding the polypeptide of SEQ ID N°6;
- SEQ ID N°7 encoding the polypeptide of SEQ ID N°8;
- SEQ ID N°9 encoding the polypeptide of SEQ ID N°10;
- SEQ ID N°11 encoding the polypeptide of SEQ ID N°12;
- SEQ ID N°21 encoding the polypeptide of SEQ ID N°22;

and fragments thereof.

19. A recombinant vector comprising a nucleic acid sequence selected among nucleic acids according to any of claims 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13 and 14.

20. The recombinant vector of claim 19 consisting of vector named X229 introduced into the recombinant *Escherichia coli* deposited at the CNCM on February 18th, 2002 under N° I-2799.

21. A recombinant cell comprising a nucleic acid sequence selected among nucleic acids according to any of claims 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13 and 14 or a vector according to claim 19 or 20.

22. The recombinant cell according to claim 21 consisting of the *Escherichia coli* deposited at the CNCM on February 18th, 2002 under N° I-2799.

23. A method for the discriminatory detection and identification of :

- *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome; versus,

- *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* in a biological sample,

comprising the following steps:

a) isolation of the DNA from the biological sample to be analyzed or production of a cDNA from the RNA of the biological sample,

b) detection of the nucleic acid sequences of the mycobacterium present in said biological sample,

c) analysis for the presence or the absence of a nucleic acid fragment according to any of claims 6, 7 and 8.

24. The method as claimed in claim 23, wherein the detection of the mycobacterial DNA sequences is carried out using nucleotide sequences complementary to said DNA sequences.

25. The method as claimed in claim 23 or 24, wherein the detection of the mycobacterial DNA sequences is carried out by amplification of these sequences using primers.

26. The method as claimed in claim 25, wherein the primers have a nucleotide sequence chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18.

27. A method for the discriminatory detection and identification of :

- *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome; versus,

- *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis* BCG in a biological sample,

comprising the following steps:

a) bringing the biological sample to be analyzed into contact with at least one pair of primers as defined in claim 25 or 26, the DNA contained in the sample having been, where appropriate, made accessible to the hybridization beforehand,

b) amplification of the DNA of the mycobacterium,

c) visualization of the amplification of the DNA fragments.

28. A kit for the discriminatory detection and identification of :
    - *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome; versus,
    - *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* in a biological sample,

comprising the following elements:

a) at least one pair of primers as defined in claim 25 or 26,
b) the reagents necessary to carry out a DNA amplification reaction,
c) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

29. The use of at least one pair of primers as defined in claim 25 or 26 for the amplification of a DNA sequence from *Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis* or *Mycobacterium bovis* BCG.

30. The use of at least one pair of primers or at least one nucleic acid fragment according to any of claims 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 for the detection of a DNA sequence from *Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium canettii, Mycobacterium microti, Mycobacterium bovis* or *Mycobacterium bovis* BCG.

31. A product of expression of all or part of the nucleic acid fragment as claimed in any of claims 6, 7 and 8.

32. A method for the *in vitro* discriminatory detection of antibodies directed against *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome, versus antibodies directed against *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG,* in a biological sample, comprising the following steps:

a) bringing the biological sample into contact with at least one product as defined in claim 31,

b) detecting the antigen-antibody complex formed.

33. A method for the *in vitro* discriminatory detection of a vaccination with *Mycobacterium bovis* BCG, an infection by *M. bovis*, *M. canettii*, *M. microti*, *M. africanum* or *M. tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome versus an infection by *Mycobacterium tuberculosis,* excepted *Mycobacterium Tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome in a mammal, comprising the following steps :

a) preparation of a biological sample containing cells, more particularly cells of the immune system of said mammal and more particularly T cells,

b) incubation of the biological sample of step a) with at least one product as defined in claim 31,

c) detection of a cellular reaction indicating prior sensitization of the mammal to said product, in particular cell proliferation and/or synthesis of proteins such as gamma-interferon.

34. A kit for the *in vitro* discriminatory diagnosis of a vaccination with *M. bovis* BCG, an infection *by M. bovis*, *M. canettii*, *M. microti*, *M. africanum* versus an infection by *M. tuberculosis* excepted by strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome, in a mammal comprising :

a) a product as defined in claim 31,

b) where appropriate, the reagents for the constitution of the medium suitable for the immunological reaction,

c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction,

d) where appropriate, a reference biological sample (negative control) free of antibodies recognized by said product,

e) where appropriate, a reference biological sample (positive control) containing a predetermined quantity of antibodies recognized by said product.

35. A mono- or polyclonal antibody, a chimeric fragment or a chimeric antibody thereof, characterized in that it is capable of specifically recognizing a product as defined in claim 31.

36. A method for the *in vitro* discriminatory detection of the presence of an antigen of *Mycobacterium tuberculosis* excepted of strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome versus an antigen of *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG* or *Mycobacterium tuberculosis* having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome in a biological sample comprising the following steps :

a) bringing the biological sample into contact with an antibody as claimed in claim 35,

b) detecting the antigen-antibody complex formed.

37. A kit for the *in vitro* discriminatory detection of the presence of an antigen of *Mycobacterium tuberculosis* excepted of strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome versus an antigen of *Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microti, Mycobacterium bovis, Mycobacterium bovis BCG,* or *Mycobacterium tuberculosis* having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome, in a biological sample comprising the following steps :

a) an antibody as claimed in claim 35,

b) the reagents for constituting the medium suitable for the immunological reaction,

c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction.

38. An immunogenic composition, characterized in that it comprises at least one product as defined in claim 31.

39. A vaccine, characterized in that it comprises at least one product as defined in claim 31 in combination with a pharmaceutically compatible vehicle and, where appropriate, one or more appropriate immunity adjuvants.

40. An *in vitro* method for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome in a biological sample, comprising the following steps :
    a) isolation of the DNA from the biological sample to be analyzed or production of a cDNA from the RNA of the biological sample,
    b) detection of the nucleic acid sequences of the mycobacterium present in said biological sample,
    c) analysis for the presence or the absence of a nucleic acid fragment according to any of claims 6, 7 and 8.

41. An *in vitro* method for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome in a biological sample, comprising the following steps:
    a) bringing the biological sample to be analyzed into contact with at least one pair of primers selected among nucleic acids according to any of claims 1 to 14, 17 and 18, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18, the DNA contained in the sample having been, where appropriate, made accessible to the hybridization beforehand,
    b) amplification of the DNA of the mycobacterium,
    c) visualization of the amplification of the DNA fragments.

42. A kit for the detection and identification of *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome, in a biological sample, comprising the following elements :
    a) at least one pair of primers selected among nucleic acids according to any of claims 1 to 14, 17 and 18, and more preferably selected among the primers

chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

b) the reagents necessary to carry out a DNA amplification reaction,

c) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

43. A method for the *in vitro* detection of antibodies directed against *Mycobacterium tuberculosis* excepted *Mycobacterium tuberculosis* strains having the sequence CTG at codon 463 of gene *katG* and having no or very few IS6110 sequences inserted in their genome, in a biological sample, comprising the following steps :

a) bringing the biological sample into contact with at least one product as defined in claim 31,

b) detecting the antigen-antibody complex formed.

44. Use of TbD1 deletion as a genetic marker for the differentiation of *Mycobacterium* strains of *Mycobacterium* complex.

45. Use of mmpL6^{551} polymorphism as a genetic marker for the differentiation of *Mycobacterium* strains of *Mycobacterium* complex.

46. Use of the genetic marker according to claim 44 in association with at least one genetic markers selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, RD11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, katG^{463}, gyrA^{95}, oxyR'^{285}, pncA^{57}, mmpL6^{551}, the specific insertion element of *M. canettii* for the differentiation of *Mycobacterium* strains of *Mycobacterium* complex.

47. An *in vitro* method for the detection and identification of *Mycobacteria* from the *Mycobacterium* complex in a biological sample, comprising the following steps :

c) analysis for the presence or the absence of a nucleic acid fragment of a sequence according to claim 6, 7 or 8, and

d) analysis of at least one additional genetic marker selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, RD11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, katG^{463}, gyrA^{95}, oxyR'^{285}, pncA^{57}, mmpL6^{551}, the specific insertion element of *M. canettii*.

48. The *in vitro* method of claim 47 wherein two additional markers are used, preferably RD4 and RD9.

49. The *in vitro* method of claim 47 wherein three additional markers are used, preferably RD4, RD9 and RD12.

50. The method according to claim 47 wherein the analysis is performed by a technique selected among sequence hybridization, nucleic acid amplification, antigen-antibody complex.

51. A kit for the detection and identification of *Mycobacteria* from the *Mycobacterium* complex in a biological sample comprising the following elements :

a) at least one pair of primers selected among nucleic acids according to any of claims 1 to 14, 17 and 18, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

b) at least one pair of primers specific of the genetic markers selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, RD11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, $katG^{463}$, $gyrA^{95}$, $oxyR'^{285}$, $pncA^{57}$, $mmpL6^{551}$, the specific insertion element of *M. canettii*

c) the reagents necessary to carry out a DNA amplification reaction,

d) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

52. A kit according to claim 51 comprising the following elements :

a) at least one pair of primers selected among nucleic acids according to any of claims 1 to 14, 17 and 18, and more preferably selected among the primers chosen from the group comprising SEQ ID N°13, SEQ ID N°14, SEQ ID N°15, SEQ ID N°16, SEQ ID N°17, SEQ ID N°18,

b) one pair of primers specific of the genetic marker RD4,

c) one pair of primers specific of the genetic marker RD9,

d) the reagents necessary to carry out a DNA amplification reaction,

e) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

53. An immunogenic composition, characterized in that it comprises the polypeptide of sequence SEQ ID N°22.

54. A vaccine, characterized in that it comprises the polypeptide of sequence SEQ ID N°22 in combination with a pharmaceutically compatible vehicle and, where appropriate, one or more appropriate immunity adjuvants.

55. Use of the genetic marker according to claim 45 in association with at least one genetic markers selected among RD1, RD2, RD3, RD4, RD5, RD6, RD7, RD8, RD9, RD10, RD11, RD13, RD14, RvD1, RvD2, RvD3, RvD4, RvD5, TbD1, $katG^{463}$, $gyrA^{95}$, $oxyR'^{285}$, $pncA^{57}$, the specific insertion element of *M. canettii* for the differentiation of Mycobacterium strains of *Mycobacterium* complex.

56. A nucleic acid specifically present in strains of *M. canettii* and absent from all other members of the *Mycobacterium* complex and having the sequence from position 399 to position 2378 of SEQ ID N°19.

57. Use of the nucleic acid according to claim 53 as a genetic marker for the differentiation of *Mycobacterium* strains of *Mycobacterium* complex.

58. A reagent for the identification of a Mycobacterium infection comprising at least polynucleotide sequences capable to hybridize under stringent conditions with at least 8 to 20 nucleotides of the RD1, RD4, RD9 and TbD1 genetic markers.

59. A reagent for the identification of a Mycobacterium infection comprising at least one polypeptide encoded by each of the RD1, RD4, RD9 and TbD1 genetic markers capable to react with an antibody or an immune serum raised against the same immunogenic molecules or fragments thereof.